MGMT Promoter Methylation Is a Strong Prognostic Biomarker for Benefit from Dose-Intensified Temozolomide Rechallenge in Progressive Glioblastoma: The DIRECTOR Trial

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Abstract: PURPOSE Rechallenge with temozolomide (TMZ) at first progression of glioblastoma after temozolomide chemoradiotherapy (TMZ/RT→TMZ) has been studied in retrospective and single-arm prospective studies, applying temozolomide continuously or using 7/14 or 21/28 days schedules. The DIRECTOR trial sought to show superiority of the 7/14 regimen. PATIENTS AND METHODS Patients with glioblastoma at first progression after TMZ/RT→TMZ and at least two maintenance temozolomide cycles were randomized to Arm A [one week on (120 mg/m(2) per day)/one week off] or Arm B [3 weeks on (80 mg/m(2) per day)/one week off]. The primary endpoint was median time-to-treatment failure (TTF) defined as progression, premature temozolomide discontinuation for toxicity, or death from any cause. O(6)-methylguanine DNA methyltransferase (MGMT) promoter methylation was prospectively assessed by methylation-specific PCR. RESULTS Because of withdrawal of support, the trial was prematurely closed to accrual after 105 patients. There was a similar outcome in both arms for median TTF [A: 1.8 months; 95% confidence intervals (CI), 1.8-3.2 vs. B: 2.0 months; 95% CI, 1.8-3.5] and overall survival [A: 9.8 months (95% CI, 6.7-13.0) vs. B: 10.6 months (95% CI, 8.1-11.6)]. Median TTF in patients with MGMT-methylated tumors was 3.2 months (95% CI, 1.8-7.4) versus 1.8 months (95% CI, 1.8-2) in MGMT-unmethylated glioblastoma. Progression-free survival rates at 6 months (PFS-6) were 39.7% with versus 6.9% without MGMT promoter methylation. CONCLUSIONS Temozolomide rechallenge is a treatment option for MGMT promoter-methylated recurrent glioblastoma. Alternative strategies need to be considered for patients with progressive glioblastoma without MGMT promoter methylation. Clin Cancer Res; 1-8. ©2015 AACR.

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DIRECTOR trial for recurrent glioblastoma

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Potential conflict of interest

J.S. and U.H. have received honoraria from Medac, G.T., M.W. and J.P. have received honoraria from MSD, J.T., M.W. and G.R. have received honoraria from Merck Serono, P.H. has provided expert testimony to Medac, W.W. has participated in a speaker's bureau for MSD, W.W., M.W. and J.P. have received research funding from MSD, M.W. has received research funding from Merck Serono, W.W. has received research funding from Boehringer, R.S. and M.W. have a consultant relationship with MSD, J.T. and M.W. have a consultant relationship with Merck Serono.

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Statement of clinical relevance

The prospective randomized DIRECTOR trial assessed the efficacy and tolerability of two different regimens of rechallenge with intensified temozolomide (TMZ) at first progression of glioblastoma after temozolomide chemoradiotherapy (TMZ/RT→TMZ). Efficacy was similar in both arms, but depended strongly on *MGMT* promoter methylation status. TMZ rechallenge should no longer be considered for patients with tumors lacking *MGMT* promoter methylation, but is an appropriate option for patients with glioblastoma harboring *MGMT* promoter methylation at first relapse.

Abstract

Purpose

Rechallenge with temozolomide (TMZ) at first progression of glioblastoma after temozolomide chemoradiotherapy (TMZ/RT→TMZ) has been studied in retrospective and single-arm prospective studies, applying TMZ continuously or using 7/14 or 21/28 days schedules. The DIRECTOR trial sought to show superiority of the 7/14 regimen.

Patients & Methods

Patients with glioblastoma at first progression after TMZ/RT→TMZ and at least 2 maintenance TMZ cycles were randomized to Arm A (one week on (120 mg/m^2 per day) / one week off) or Arm B (three weeks on (80 mg/m^2 per day) / one week off). The primary end point was median time to treatment failure (TTF) defined as progression, premature TMZ discontinuation for toxicity, or death from any cause. O^6-methylguanine DNA methyltransferase (*MGMT)* promoter methylation was prospectively assessed by methylation-specific PCR.

Results

Because of withdrawal of support, the trial was prematurely closed to accrual after 105 patients. There was a similar outcome in both arms for median TTF (A: 1.8 months [95% CI 1.8-3.2] versus B: 2.0 months [95% CI 1.8-3.5]) and overall survival (OS) (A: 9.8 months [95% CI 6.7-13.0] versus B: 10.6 months [95% CI 8.1-11.6]). Median TTF in patients with *MGMT*-methylated tumors was 3.2 months [95% CI 1.8-7.4] versus 1.8 months [95% CI 1.8-2] in *MGMT*-unmethylated glioblastoma. Progression-free survival rates at six months (PFS-6) were 39.7% with versus 6.9% without *MGMT* promoter methylation.

Conclusions

TMZ rechallenge is a treatment option for *MGMT* promoter-methylated recurrent glioblastoma. Alternative strategies need to be considered for patients with progressive glioblastoma without *MGMT* promoter methylation.

Introduction

The standard of care for newly diagnosed glioblastoma, with an incidence of more than 3/100,000 the most common primary malignant brain tumor, includes resection or biopsy as feasible, involved field radiotherapy, and concomitant and adjuvant temozolomide (TMZ/RT→TMZ) (1). While anti-angiogenic agents such as the antibody to vascular endothelial growth factor, bevacizumab, or the integrin inhibitor cilengitide failed to prolong overall survival (2-4), the novel approach of tumor-treating fields provided a survival advantage and may be incorporated into the future first-line treatment (5).

Standards of care at progression are less well defined (6): Depending on approval status, individual patient and tumor factors, and local preference, the most commonly used systemic therapeutic approaches include nitrosoureas such as lomustine (CCNU) which has become the standard of care in randomized clinical trials for recurrent glioblastoma (7, 8), temozolomide rechallenge using various regimens (9-12), and bevacizumab (13, 14). The need for prospective assessment of TMZ rechallenge after systematic recognition of pseudoprogression as a potential confounder of second-line treatments (15, 16) and the controversy regarding the optimal dosing of TMZ for patients with recurrent glioblastoma after failure of first-line TMZ/RT→TMZ led to the design of the DIRECTOR (***D****ose-****I****ntensified* ***Rec****hallenge with* ***T****emozolomide,* ***O****ne Week on One Week Off versus Three Weeks on One Week*

*Off in Patients with Progressive or **R**ecurrent Glioblastoma*) trial, which sought to explore the activity of two widely used regimens of dose-intense TMZ for recurrent glioblastoma, one week on / one week off (7/14) (9) *versus* three weeks on / one week off (10).

Patients and Methods

Study design

DIRECTOR (NCT 00941460) was designed as a prospective, open-label, randomized, 2-arm trial of two competing TMZ dosing regimens for patients with glioblastoma at first relapse or progression. The primary objective was to show the superiority of Arm A (one week on / one week off) over Arm B (three weeks on / one week off), the primary endpoint being time to treatment failure (TTF). Major inclusion criteria were: progressive or recurrent glioblastoma documented by MRI no earlier than 180 days after first surgery for glioblastoma and no earlier than 90 days after completion of radiotherapy (patients with progression outside the radiation field were also not allowed to be entered into the trial unless these time frames were respected); histological diagnosis of glioblastoma; tissue available for the determination of O^6-methylguanine DNA methyltransferase (*MGMT)* promoter methylation in the primary or in the recurrent tumor; prior treatment with TMZ/RT and at least two cycles of maintenance TMZ (5/28); informed consent; age 18-80 years; Karnofsky performance score $\geq$ 50%; absolute neutrophil counts > 1,500/µl; platelet counts > 100,000/µl; hemoglobin > 10 g/dl; serum creatinin < 1.5-fold upper normal range; ASAT or ALAT < 3-fold upper normal range unless attributed to anticonvulsants; alkaline phosphatase < 3-fold upper normal range; women with

childbearing potential must have a negative serum pregnancy test ≤14 days prior to study enrollment. Obligatory *MGMT* testing of the recurrent tumor as opposed to the primary tumor tissue was initially required, but no longer requested when it became clear that the result of the *MGMT* status determination rarely changes in the course of disease (17). All patients gave written informed consent, and the study was approved by the local ethical committees and competent authorities. The trial was prematurely closed for withdrawal of support after the merger of Schering Plough (Kenilworth, NJ) with Merck, Sharp & Dohme (Whitehouse Station, NJ). Databank closure was on June 30, 2013.

Central pathology review, DNA extraction and MGMT promoter methylation analysis

All tissue samples from primary or recurrent tumor were confirmed by central pathology review (G.R.) to represent glioblastoma according to the World Health Organisation (WHO) classification of tumors of the central nervous system (18). Tumor DNA was extracted from formalin-fixed and paraffin-embedded tissue samples using the Qiagen blood and tissue DNA extraction kit (Qiagen, Hilden, Germany). Each tumor sample used for DNA extraction was histologically verified to contain vital glioblastoma tissue with an estimated tumor cell content $\geq$ 80%. The *MGMT* promoter methylation status was determined by methylation-specific PCR (MSP) and evaluated as reported (19). This MSP assays had been used in several previous studies (20-22) and was proven to show high concordance with results obtained by the MSP assay of MDxHealth S.A. (Herstal, Belgium) (22) and DNA pyrosequencing (17).

Study Treatment

Patients were allocated either to the one week on / one week off regimen (7/14, Arm A) or to the three weeks on / one week off regimen (21/28, Arm B) of dose-intensified TMZ using a treatment allocation algorithm (23) with a probability of a minimizing allocation set at 0.9. Arm A patients were treated at an initial dose of 120 mg/m^2 unless there had been grade III or IV myelotoxicity with conventional TMZ (5/28) previously. These patients were started at 90 mg/m^2. TMZ was given orally on days 1-7 and 15-21. Arm B patients started with an initial dose of 80 mg/m^2 unless there had been significant myelotoxicity with conventional TMZ (5/28) previously. These patients started at 60 mg/m^2. TMZ was given orally on days 1-21. A treatment cycle was defined as two completed weeks of TMZ within four weeks in Arm A and as three weeks of continuous TMZ within four weeks in Arm B. Dose modifications were foreseen according to hematological parameters as outlined in Supplementary Table 1.

Assessments and endpoints

Patients were to be seen weekly during cycle 1 and monthly thereafter for general evaluation and blood tests. Toxicity was evaluated using the Common Terminology Criteria for Adverse Events (CTCAE v3.0). Cognitive function was assessed by Mini-Mental State Examination (MMSE) in 4-weekly intervals. Quality of life was monitored by EORTC QLQ-C30 and QLQ-BN20 in 8-weekly intervals. Disease status was monitored by MRI in 8-weekly intervals and assessed using Macdonald criteria as prespecified in the protocol (24). The primary endpoint, TTF, was calculated from randomization to any of the following: progressive disease defined by Macdonald criteria (24), death for any reason, or toxicity leading to discontinuation of study

treatment for any reason. Secondary endpoints included progression-free survival (PFS) calculated as the time from randomization to the first documented evidence of progression of disease, survival from randomization, and efficacy parameters in subgroups defined by *MGMT* status. Radiological progression was evaluated at each center and also centrally verified post-hoc (blindly to previous results).

Statistical analyses

The targeted sample size was 83 patients per arm, and no interim analysis was planned. This size would have allowed for a detection of an improvement in median TTF from 18.2 weeks for Arm B to 29.2 weeks for Arm A (hazard ratio 0.63) (10, 25). Based on these assumptions, there was approximately 80% power to detect the stated difference in TTF between the two treatment arms for a two-sided level of 0.05. Treatment arms were compared using a permutation test (26) with 9999 replicates in the Cox proportional hazard model with the same parameters as explanatory variables used for the treatment allocation algorithm (*MGMT* promoter methylation status, >2 months since previous TMZ treatment, age at least 50 years, KPS 50-60, 70-80 or 90-100) along with the variable for treatment. Since p values from permutation tests were sufficiently close to those based on partial likelihoods as routinely used in parameter estimation in the Cox model, the latter were used for all subgroup analyses. Secondary analyses with respect to time-to-event variables were done using Cox proportional hazards models when considering multiple explanatory variables. Log-rank or bootstrap tests to compare median times and parametric tests with standard error approximation were used for univariate tests, especially when comparing rates at fixed time points. With respect to categorical variables, we used Fisher's exact test or logistic regression, and with respect to continuous variables linear regression models. Specifically, quality of life was analyzed using a linear

mixed regression model with patient as random effect. All p values, statistical tests and confidence intervals beyond the analysis of the primary criterion were not corrected for multiplicity and are to be interpreted as exploratory.

Results

Patient characteristics

105 patients were randomized at 16 sites from 9/2009 to 6/2012. Table 1 summarizes patient characteristics per treatment arm. Arms A and B were overall well balanced. More patients in Arm B had surgery for recurrent disease whereas more patients in Arm A had steroids at study entry. At the time of databank closure (30 June 2013), 87 deaths were documented, 84 were attributed to tumor progression, 3 documented with unknown course. No patient was still on study treatment. Four patients had not reached the primary endpoint of TTF (Figure 1).

Safety and tolerability

All adverse events were categorized by system organ class and graded according to CTCAE. There was no relevant difference between both arms regarding the frequency and severity of adverse events in the hematological system. Profound lymphopenia was the most common hematological toxicity, 19% in Arm A and 29% in Arm B (Supplementary Table 1). Severe infections, however, were rare. Non-hematalogical adverse events, e.g., disorders of the gastrointestinal system, nervous system, metabolism, respiratory system, skin, cardiovascular system or musculoskeletal system occurred at similar rates in both treatment arms and were overall infrequent.

Outcome by treatment and MGMT status

All clinical outcome parameters were comparable in Arms A and B (Figure 2, Table 2). Median TTF was below 2 months whereas median OS from first intake of study drug was in the range of 10 months. The p value from the permutation test (p=0.488) was close enough to the p value using partial likelihood from the Cox model (p=0.485) to justify taking the latter one for all other analyses. There were 2 CR (4%) and 2 PR (4%) in Arm A and 4 CR (8%) and 4 PR (8%) in Arm B by local assessment (p=0.68) in response to the study treatment. The median duration of the 6 CR was 4.5 months (95% CI 1.8-11.0). The median duration of the 6 PR was 3 months (95% CI 1.8-13.6). TTF was diagnosed because of PD in all but three patients, confirming that tolerability was good. One patient developed wound infection at day 26, necessitating TMZ discontinuation, 2 patients died without documented PD. Age was not prognostic. As required per protocol *MGMT* status from primary or recurrent tumor was available for all patients. *MGMT* promoter methylation was strongly associated with superior TTF and all other outcome parameters (Figure 2, Table 3). The TTF difference between patients with versus without *MGMT* promoter methylation was more prominent in Arm B than in Arm A (Supplementary table 2). Overall survival from initial histological diagnosis of glioblastoma was 25.4 months (95% CI 17.8-32.3) in Arm A and 22.7 months (95% CI 18.5-27.2) in Arm B. This shows that patients enrolled into randomized trials for recurrent glioblastoma represent a selected population.

Central radiology review

Serial MRI of 85 patients were available for post-hoc central review of progression. All these patients had measurable disease at baseline. The time point of progression

was centrally confirmed in 81 patients. It was antedated 1 scan in 2 patients and not confirmed in 2 patients; 0 of 1 CR and 2 of 3 PR were confirmed. Insufficient scans were provided for the other 12 patients considered objective responders locally.

Outcome by preexposure to TMZ

We also separated the patient populations by intensity and interval of preexposure to TMZ. Administration of more than 6 cycles of maintenance TMZ is uncommon in Europe (Table 1). To this end, we compared patients with intervals below (n=40) or above 2 months (n=65) since their last TMZ intake as specified in the study protocol. Four of 6 CR and all 6 PR were noted in the latter group. Further, there was significantly improved outcome in patients with a longer delay since the last administration of TMZ, more prominent in Arm A than in Arm B and largely confined to patients with *MGMT* promoter methylation (Supplementary table 3).

MMSE and quality of life

Serial assessments of MMSE and quality of life using EORTC QLQ-C30 and QLQ-BN20 were grouped into (i) pre-treatment, (ii) during study treatment and (iii) after study treatment assessments. For the latter two time intervals carrying multiple measures, we determined patient-wise minimum, median and maximum scores. The MMSE as a surrogate measure of cognitive function remained stable during treatment and did not exhibit a decline after the end of study treatment as long as data were captured (Supplementary Figure 1, Supplementary table 4). There was relatively little difference in quality of life assessed by QLQ-C30 and QLQ-BN20 when compared after the first 90 days of study treatment (Supplementary table 5). Treatment-by-time interaction indicated that quality of life developments were somewhat more favorable in Arm B, with significant differences for pain

(Supplementary table 6). Although most scales are deteriorating over time (positive slope terms in either arm), the lack of major decline over time may result from the low number of assessments after the end of study treatment (Supplementary Table 7).

Multivariate modeling of outcome

Cox proportional hazards modeling for TTF revealed *MGMT* promoter methylation status and time interval from last TMZ exposure as independent prognostic factors whereas no such role was identified for age, KPS, surgery for recurrent tumor prior to enrolment (Table 4). Steroid administration at study entry, body surface area, body weight, red or white blood cell or lymphocyte counts, hemoglobin or hematocrit at study entry were not prognostic for TTF (data not shown). Similar results were obtained when Cox proportional hazards modeling was applied to PFS whereas only *MGMT* status was prognostic for survival from first study drug administration (data not shown).

Discussion

Standards of care in recurrent glioblastoma are not well defined. This definitive report of the phase II randomized DIRECTOR trial indicates that TMZ rechallenge is a valid treatment option for patients with recurrent glioblastoma with, but not without, *MGMT* promoter methylation.

The optimal dosing of TMZ in glioblastoma became a dominant topic in the first decade of this century in Neuro-Oncology, in part reflecting the lack of promising alternative drugs, in part also reflecting the consideration that TMZ activity is critically limited by chemoresistance afforded by *MGMT* (27, 28). *MGMT* promoter methylation

is observed in 30-40% of glioblastomas, presumably resulting in decreased *MGMT* gene expression in the *MGMT* promoter-methylated tumor cells, thereby rendering glioblastomas more sensitive to TMZ. However, a predictive role of *MGMT* promoter methylation for benefit from alkylating chemotherapy including TMZ has only been defined for glioblastoma whereas *MGMT* promoter methylation is prognostic for better outcome with either radiotherapy or chemotherapy in patients with anaplastic gliomas (29, 30). This difference in biological significance of *MGMT* promoter methylation is probably not related to grade of malignancy *per se*, but to the differential distribution of isocitrate dehydrogenase (*IDH*) mutations among these tumors. *MGMT* promoter methylation associated with *IDH* mutation and the glioma-associated CpG island methylator phenotype (G-CIMP) does not have the same significance as MGMT promoter methylation on the wild-type *IDH* background of glioblastoma (31, 32).

Since TMZ depletes MGMT protein in peripheral blood mononuclear cells (33) and presumably glioblastoma cells, too, it was tempting to speculate that higher doses of TMZ given over a longer time frame would eventually deplete MGMT. Accordingly, it was assumed that specifically patients with glioblastomas lacking *MGMT* promoter methylation might benefit from dose-intense TMZ regimens. In addition and in parallel to DIRECTOR, two further trials explored the potential role of TMZ dose intensification in glioblastoma. For the newly diagnosed setting, the hypothesis that more TMZ might deplete MGMT and confer a survival benefit was falsified by the RTOG 0525 trial which confirmed the strong prognostic role of the *MGMT* status in TMZ-treated patients, but showed no difference between standard-dosed TMZ or a three weeks on / one week off regimen for 6-12 cycles in the maintenance phase, also not when the analysis was stratified for *MGMT* status (34). The BR12 trial analysed the same two regimens in comparison with procarbacine, CCNU and

vincristine (PCV) in recurrent malignant glioma and similarly observed no difference between the three arms (35). However, this trial had enrolled chemonaive patients with WHO grade III or IV gliomas which does not inform about the current situation in clinical practice where recurrent or progressive glioblastoma patients have commonly been pretreated with TMZ/RT→TMZ.

The DIRECTOR trial reports a median TTF in the range of 2 months and yields overall no evidence that there are clinically relevant differences between the two dosing regimens, regarding either efficacy, safety or tolerability (Figure 2. Tables 2 and 3). Importantly, the dosing regimens were both confirmed to be feasible, given that PD was driving TTF in all, but one patient(s). The PFS-6 rate of 21% is in the range of previously reported figures of 11-24% (11, 12, 36). In contrast to the RESCUE trial (11), we observed a better PFS in patients off TMZ for 2 months or more (Supplementary table 3). Of note, it is uncommon in Europe to give TMZ for more than 6 months (Table 1). These considerations indicate that some of the patients escalated to dose-intensified TMZ regimens early in the disease course in RESCUE as well as in our previous reports (37) were in fact suffering from pseudoprogression, artificially raising the PFS-6 rate. Increased awareness of pseudoprogression may thus explain an apparent decrease in PFS-6 rates with TMZ rechallenge in contemporary studies (12), and challenges all cross trial comparisons to older series. Moreover, differences in the PFS-6 figures for TMZ rechallenge – and probably CCNU, too - are likely to be related to the proportion of patients with tumors with *MGMT* promoter methylation in these studies, e.g., PFS-6 was 26% with versus 0% without *MGMT* promoter methylation in the control arm of the BELOB trial (38).

The major limitations of the DIRECTOR trial are the relatively small sample size and the premature closure of the study which allows for less definitive conclusions. Yet, despite the lower than planned sample size and the premature trial closure, the

likelyhood of a major difference in efficacy between the different TMZ schedules is very low.

In fact, the most important result of DIRECTOR is the strong prognostic role of the *MGMT* promoter methylation status in patients rechallenged with TMZ that has not previously been studied prospectively in an adequately sized patient population. In contrast, age and KPS were not prognostic, likely reflecting preselection of patients enrolled into randomized trials for recurrent as opposed to newly diagnosed glioblastoma enriching patients with a similar, relatively favorable outcome. *MGMT* status was centrally assessed and was available for all patients. Although there was only a moderate advantage in median TTF of 3.2 versus 1.8 months in patients with MGMT promoter-methylated versus unmethylated tumors, PFS-6 was increased 5.8-fold, and OS at 12 months 2.4-fold (Figure 2, Table 3). Yet, given the absence of an inactive comparator or a placebo, it cannot be excluded that *MGMT* promoter methylation is merely prognostic. Thus, bevacizumab alone was associated with superior PFS at 6 months in patients with tumors with *versus* without *MGMT* promoter methylation in the BELOB trial, too (38), supporting a prognostic role of *MGMT* promoter methylation in recurrent glioblastoma. Randomization between TMZ and placebo and the demonstration of benefit from TMZ exclusively in patients with tumors with *MGMT* promoter methylation would be required for definitive confirmation, but is neither feasible nor ethical in patients with recurrent glioblastoma.

The findings of the DIRECTOR trial have implications for current clinical practice. Based on DIRECTOR, TMZ rechallenge should no longer be considered for patients with tumors lacking *MGMT* promoter methylation, but remains a viable option for patients with *MGMT* promoter-methylated glioblastomas, notably after a drug-free interval of 2 months or more. Whether TMZ given at 5 out of 28 days would be as effective as dose-intense regimens in patients recurring after a drug-free interval,

remains uncertain, but the 5/28 regimen may be preferred in that setting because of better tolerability. More importantly, it may be speculated that a similarly profound prognostic effect of the *MGMT* status would have been seen, had the patients been treated with nitrosoureas instead of TMZ (38). If confirmed, this would call for *MGMT* testing of primary or recurrent tumor and stratification for all, notably smaller randomized recurrent glioblastoma trials carrying an alkylator control arm because imbalances in the distribution of patients with *MGMT*-unmethylated *versus MGMT*-methylated tumors could severely bias outcome. In conclusion, DIRECTOR supports stratified treatment algorithms based on *MGMT* promoter methylation status in recurrent glioblastoma and advocates an alkylator regimen, including dose-dense TMZ, as the most appropriate option for patients with glioblastoma harboring *MGMT* promoter methylation.

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References

1. Weller M, van den Bent M, Hopkins K, Tonn JC, Stupp R, Falini A, et al. EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma. The Lancet Oncology. 2014;15:e395-e403.

2. Chinot OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. The New England journal of medicine. 2014;370:709-22.

3. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. The New England journal of medicine. 2014;370:699-708.

4. Stupp R, Hegi ME, Gorlia T, Erridge SC, Perry J, Hong YK, et al. Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071-22072 study): a multicentre, randomised, open-label, phase 3 trial. The lancet oncology. 2014;15:1100-8.

5. Stupp R, Wong ET, Scott CB, Kirson ED, Palti Y, Taillibert S, et al. Interim analysis of the EF-14 trial: a prospective, multicenter trial of NovoTTF-100A together

with temozolomide compared to temozolomide alone in patients with newly diagnosed GBM. Presented at: 19th Annual Meeting of the Society for Neuro-Oncology; November 13-16, 2014: Miami, FL. 2014.

6. Weller M, Cloughesy T, Perry JR, Wick W. Standards of care for treatment of recurrent glioblastoma--are we there yet? Neuro-oncology. 2013;15:4-27.

7. Wick W, Puduvalli VK, Chamberlain MC, van den Bent MJ, Carpentier AF, Cher LM, et al. Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28:1168-74.

8. Batchelor TT, Mulholland P, Neyns B, Nabors LB, Campone M, Wick A, et al. Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31:3212-8.

9. Wick W, Steinbach JP, Kuker WM, Dichgans J, Bamberg M, Weller M. One week on/one week off: a novel active regimen of temozolomide for recurrent glioblastoma. Neurology. 2004;62:2113-5.

10. Brandes AA, Tosoni A, Cavallo G, Bertorelle R, Gioia V, Franceschi E, et al. Temozolomide 3 weeks on and 1 week off as first-line therapy for recurrent glioblastoma: phase II study from gruppo italiano cooperativo di neuro-oncologia (GICNO). British journal of cancer. 2006;95:1155-60.

11. Perry JR, Belanger K, Mason WP, Fulton D, Kavan P, Easaw J, et al. Phase II trial of continuous dose-intense temozolomide in recurrent malignant glioma: RESCUE study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28:2051-7.

12. Norden AD, Lesser GJ, Drappatz J, Ligon KL, Hammond SN, Lee EQ, et al. Phase 2 study of dose-intense temozolomide in recurrent glioblastoma. Neuro-oncology. 2013;15:930-5.

13. Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, Abrey LE, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009;27:4733-40.

14. Kreisl TN, Kim L, Moore K, Duic P, Royce C, Stroud I, et al. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009;27:740-5.

15. Gerstner ER, McNamara MB, Norden AD, Lafrankie D, Wen PY. Effect of adding temozolomide to radiation therapy on the incidence of pseudo-progression. Journal of neuro-oncology. 2009;94:97-101.

16. Brandsma D, van den Bent MJ. Pseudoprogression and pseudoresponse in the treatment of gliomas. Current opinion in neurology. 2009;22:633-8.

17. Felsberg J, Thon N, Eigenbrod S, Hentschel B, Sabel MC, Westphal M, et al. Promoter methylation and expression of MGMT and the DNA mismatch repair genes MLH1, MSH2, MSH6 and PMS2 in paired primary and recurrent glioblastomas. International journal of cancer Journal international du cancer. 2011;129:659-70.

18. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumours of the central nervous system. Acta neuropathologica. 2007;114:97-109.

19. Felsberg J, Rapp M, Loeser S, Fimmers R, Stummer W, Goeppert M, et al. Prognostic significance of molecular markers and extent of resection in primary

glioblastoma patients. Clinical cancer research : an official journal of the American Association for Cancer Research. 2009;15:6683-93.

20. Weller M, Felsberg J, Hartmann C, Berger H, Steinbach JP, Schramm J, et al. Molecular predictors of progression-free and overall survival in patients with newly diagnosed glioblastoma: a prospective translational study of the German Glioma Network. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009;27:5743-50.

21. Reifenberger G, Hentschel B, Felsberg J, Schackert G, Simon M, Schnell O, et al. Predictive impact of MGMT promoter methylation in glioblastoma of the elderly. International journal of cancer Journal international du cancer. 2012;131:1342-50.

22. Wick W, Platten M, Meisner C, Felsberg J, Tabatabai G, Simon M, et al. Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. The lancet oncology. 2012;13:707-15.

23. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics. 1975;31:103-15.

24. Macdonald DR, Cascino TL, Schold SC, Jr., Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1990;8:1277-80.

25. Wick A, Felsberg J, Steinbach JP, Herrlinger U, Platten M, Blaschke B, et al. Efficacy and tolerability of temozolomide in an alternating weekly regimen in patients with recurrent glioma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2007;25:3357-61.

26. Hasegawa T, Tango T. Permutation test following covariate-adaptive randomization in randomized controlled trials. Journal of biopharmaceutical statistics. 2009;19:106-19.

27. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. The New England journal of medicine. 2005;352:997-1003.

28. Weller M, Stupp R, Reifenberger G, Brandes AA, van den Bent MJ, Wick W, et al. MGMT promoter methylation in malignant gliomas: ready for personalized medicine? Nature reviews Neurology. 2010;6:39-51.

29. Wick W, Hartmann C, Engel C, Stoffels M, Felsberg J, Stockhammer F, et al. NOA-04 randomized phase III trial of sequential radiochemotherapy of anaplastic glioma with procarbazine, lomustine, and vincristine or temozolomide. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009;27:5874-80.

30. van den Bent MJ, Dubbink HJ, Sanson M, van der Lee-Haarloo CR, Hegi M, Jeuken JW, et al. MGMT promoter methylation is prognostic but not predictive for outcome to adjuvant PCV chemotherapy in anaplastic oligodendroglial tumors: a report from EORTC Brain Tumor Group Study 26951. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009;27:5881-6.

31. Bady P, Sciuscio D, Diserens AC, Bloch J, van den Bent MJ, Marosi C, et al. MGMT methylation analysis of glioblastoma on the Infinium methylation BeadChip identifies two distinct CpG regions associated with gene silencing and outcome, yielding a prediction model for comparisons across datasets, tumor grades, and CIMP-status. Acta neuropathologica. 2012;124:547-60.

32. Wick W, Meisner C, Hentschel B, Platten M, Schilling A, Wiestler B, et al. Prognostic or predictive value of MGMT promoter methylation in gliomas depends on IDH1 mutation. Neurology. 2013;81:1515-22.

33. Tolcher AW, Gerson SL, Denis L, Geyer C, Hammond LA, Patnaik A, et al. Marked inactivation of O6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules. British journal of cancer. 2003;88:1004-11.

34. Gilbert MR, Wang M, Aldape KD, Stupp R, Hegi ME, Jaeckle KA, et al. Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31:4085-91.

35. Brada M, Stenning S, Gabe R, Thompson LC, Levy D, Rampling R, et al. Temozolomide versus procarbazine, lomustine, and vincristine in recurrent high-grade glioma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28:4601-8.

36. Han SJ, Rolston JD, Molinaro AM, Clarke JL, Prados MD, Chang SM, et al. Phase II trial of 7 days on/7 days off temozolmide for recurrent high-grade glioma. Neuro-oncology. 2014;16:1255-62.

37. Wick A, Pascher C, Wick W, Jauch T, Weller M, Bogdahn U, et al. Rechallenge with temozolomide in patients with recurrent gliomas. Journal of neurology. 2009;256:734-41.

38. Taal W, Oosterkamp HM, Walenkamp AM, Dubbink HJ, Beerepoot LV, Hanse MC, et al. Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): a randomised controlled phase 2 trial. The lancet oncology. 2014;15:943-53.

Table 1. Patient characteristics prior to enrolment

	Arm A 7/7 N=52	Arm B 21/7 N=53
Age at diagnosis		
Median (years)	58	56
Range (years)	21-62	37-59
Gender		
Male	34 (65%)	35 (66%)
Female	18 (35%)	18 (34%)
***MGMT* promoter**		
Methylated	28 (53.8%)	31 (58.5%)
Unmethylated	24 (46.2%)	22 (41.5%)
First-line therapy		
TMZ/RT	52 (100.0%)	53 (100.0%)
Number of maintenance TMZ cycles		
No data	1 (1.9%)	0 (0%)
≤3	9 (17.3%)	12 (22.6%)
4-6	32 (61.5%)	33 (62.3%)
7 or more	10 (19.2%)	8 (15.1%)
Time since last TMZ administration		
< 2 months	20 (38.5%)	20 (37.7%)
≥ 2 months	32 (61.5%)	33 (62.3%)
Survival		
Median PFS (months, 95% CI)	12.0 (8.8-17.0)	11.0 (9.2-12.9)
KPS at study entry		
90-100	30 (57.7%)	30 (56.6%)
70-80	15 (28.8%)	16 (30.2 %)
<70	7 (13.5%)	7 (13.2%)
Steroids at study entry		
Yes	16 (30.8%)	12 (22.6%)
No	36 (69.2 %)	41 (77.4%)
Surgery for recurrence		
Yes	29 (55.8%)	32 (60.4%)
No	23 (44.2%)	21 (39.6%)

Abbreviations: CI, confidence interval; KPS, Karnofsky performance score; MGMT, O^6-methylguanine DNA methyltransferase; OS, overall survival; PFS, progression-free survival; TMZ, temozolomide.

Table 2. Outcome by treatment arm.

	Arm A (7/14)			Arm B (21/28)			
	Patients	Events	Time in months (95% CI)	Patients	Events	Time in months (95% CI)	p
Median TTF	52	49	1.8 [1.8; 3.2]	53	49	1.95 [1.84; 3.44]	0.37
Median survival from first study drug administration	52	42	9.8 [6.6; 13.0]	53	45	10.6 [8.1; 11.7]	0.78
			Rate in % (95% CI)			Rate in % (95% CI)	
TTF-6	50	42	17.1 [8.2; 28.8]	52	39	25.0 [14.3; 37.3]	0.33
PFS-6	50	42	17.1 [8.2; 28.8]	52	39	25.0 [14.3; 37.3]	0.33
Survival rate at 12 months from first study drug administration	45	27	41.0 [26.7; 54.8]	49	33	32.7 [20.2; 45.9]	0.40

Abbreviations: CI, confidence interval; PFS, progression-free survival; TTF, time to treatment failure.

Table 3. Outcome by *MGMT* promoter methylation status.

	MGMT-unmethylated glioblastoma			*MGMT*-methylated glioblastoma			
	Patients	Events	Time in months (95% CI)	Patients	Events	Time in months (95% CI)	p
Median TTF	59	56	1.8 [1.8; 2.0]	46	42	3.2 [1.8; 7.3]	0.0014
Median survival from first study drug administration	59	50	7.9 [6.3; 10.3]	46	36	12.5 [9.8; 17.4]	0.0009
			Rate in % (95% CI)			Rate in% (95% CI)	
PFS-6	58	54	6.9 [2.2; 15.3]	44	27	39.7 [25.5; 53.5]	<0.0001
Survival rate at 12 months from first study drug administration	53	41	22.9 [12.7; 34.9]	41	19	54.1 (37.8; 67.8)	0.0013

Abbreviations: CI, confidence interval; PFS, progression-free survival; TTF, time to treatment failure.

Table 4. Multivariate analyses of predictors for inferior TTF.

	Hazard ratio and 95% CI	**p**
Arm A versus Arm B	1.16 (0.76-1.76)	0.485
Age at study entry 50+ versus 18-49 years	1.27 (0.76-2.20)	0.381
Time interval since last TMZ: < versus ≥ 2 months	1.60 (1.00-2.55)	**0.036**
Salvage surgery: no versus yes	1.02 (0.65-1.57)	0.945
KPS 50-60 versus 90-100 KPS 70-80 versus 90-100	1.03 (0.52-1.92) 1.05 (0.63-1.73)	0.786 0.841
MGMT promoter: unmethylated versus methylated	1.76 (1.11-2.82)	**0.017**

[1]Hazard ratios as exponential function of parameter estimates and confidence interval. Estimates from a Cox model containing arm, treatment, age, time since last TMZ, salvage surgery: no versus yes, KPS, and *MGMT* promoter methylation status as explanatory variables. Other factors which are mentioned in the text, were included as additional variables in turn (one at a time).

Figure Legends

Figure 1. **CONSORT chart.**

Figure 2. **Clinical outcome.** TTF (A) and OS (B) in Arm A (7/14) versus Arm B (21/28). TTF (C) and OS (D) in patients without *versus* with *MGMT* promoter methylation.

MGMT promoter methylation is a strong prognostic biomarker for benefit from dose-intensified temozolomide rechallenge in progressive glioblastoma: the DIRECTOR trial

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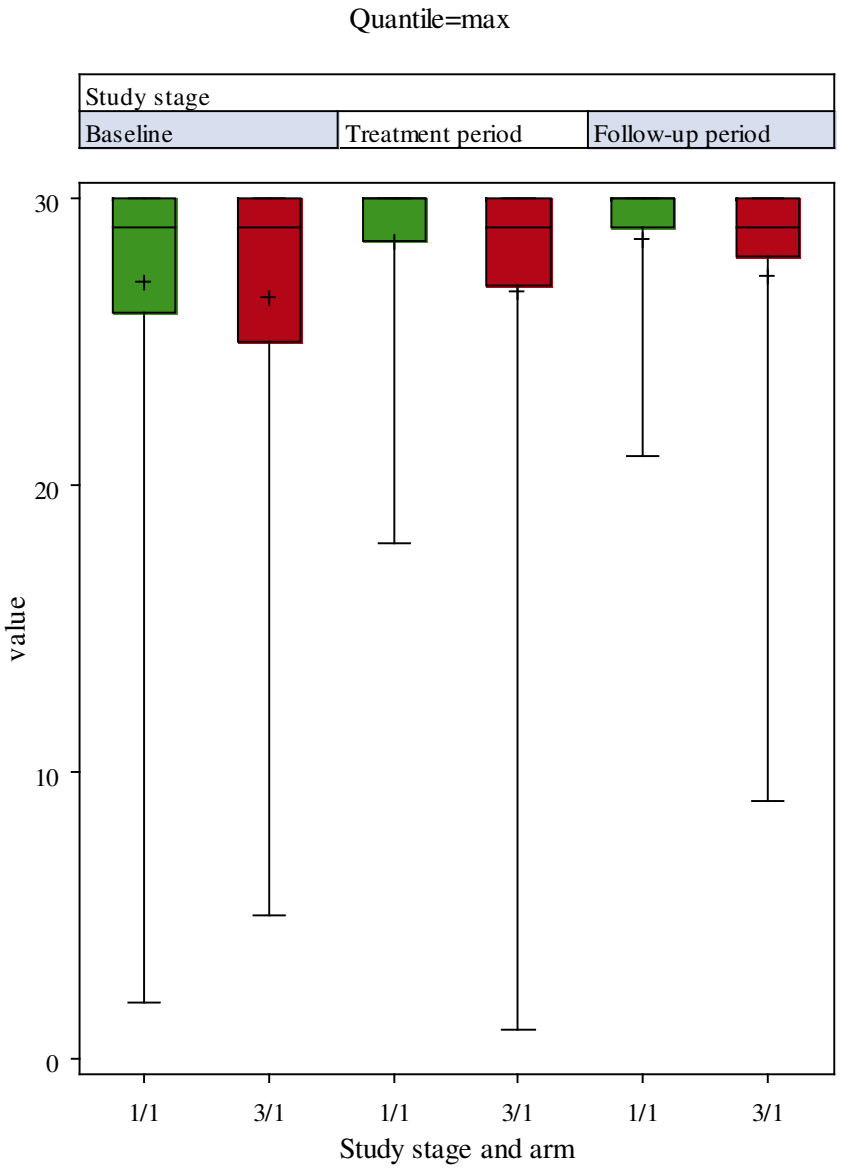
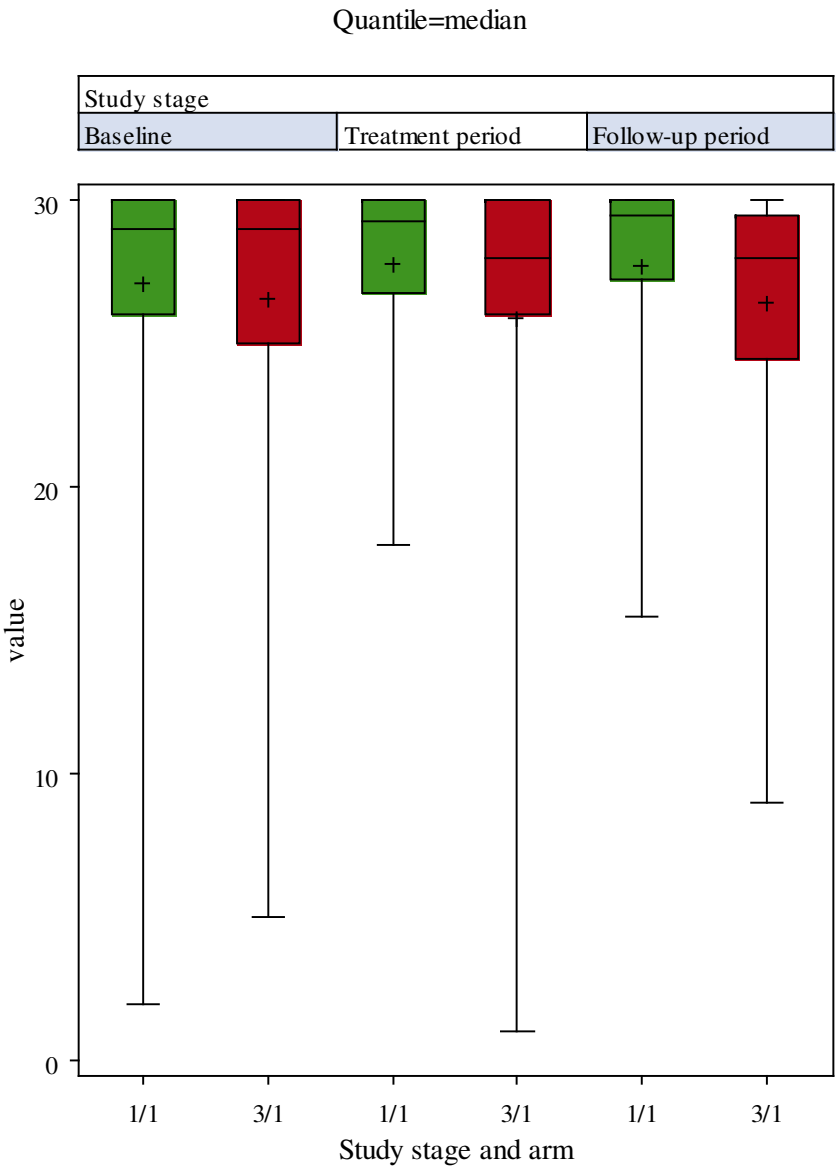
DIRECTOR trial for recurrent glioblastoma

Supplementary Figure 1. MMSE assessment at study entry, during treatment, and at follow-up. Arm a is depicted in green, Arm B in red.

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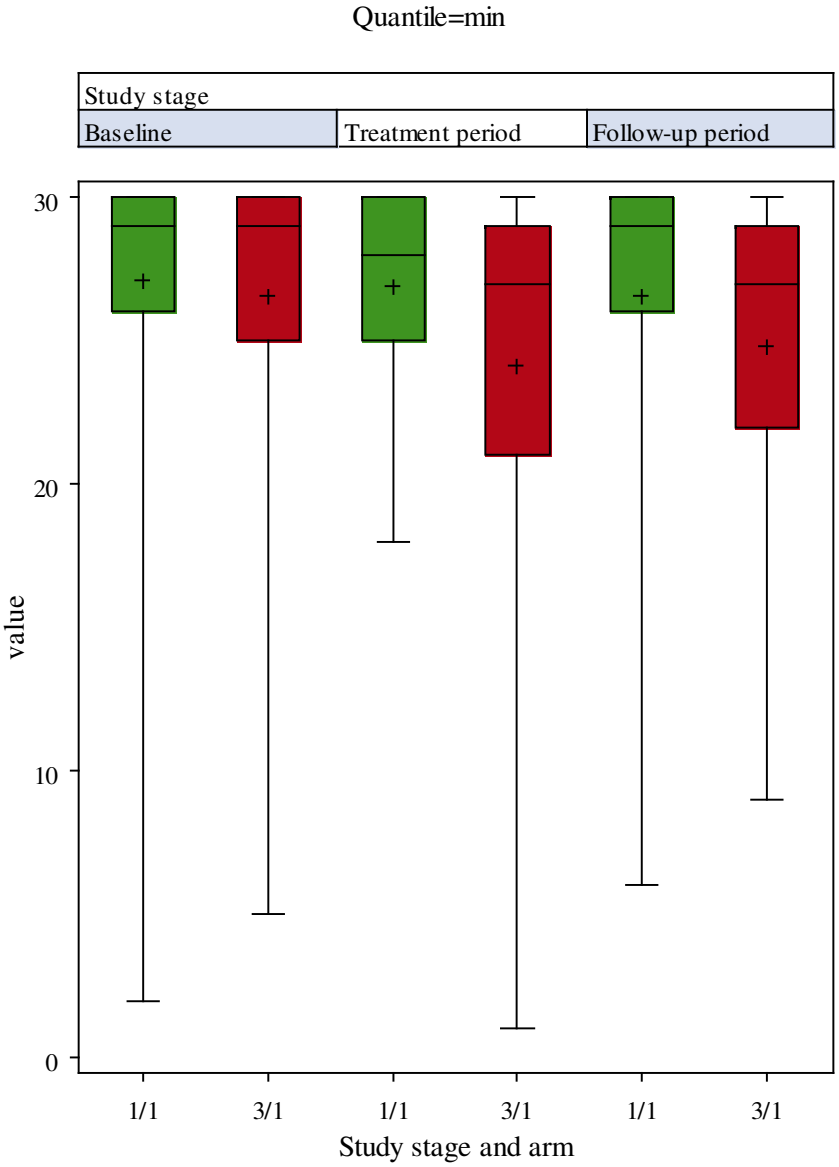
Study stage and arm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Study stage and arm

MGMT promoter methylation is a strong prognostic biomarker for benefit from dose-intensified temozolomide rechallenge in progressive glioblastoma: the DIRECTOR trial

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DIRECTOR trial for recurrent glioblastoma

Supplementary Tables

Supplementary Table 1. Safety and tolerability.

	Arm A n = 52			Arm B n = 52*		
CTCAE grade	**2**	**3**	**4**	**2**	**3**	**4**
Hematological toxicity[1], n						
Neutropenia	0	1	0	0	1	0
Lymphopenia	3	8	2	2	14	1
Thrombocytopenia	1	1	1	1	2	0
Liver enzyme elevation (γ-GT), n	0	0	1	1	0	0
All infections[2], n	5	5	0	5	4	0
Thrombembolic event, n	4	2	0	1	0	0
Fatigue, n	4	0	0	4	1	0
Nausea / Vomiting[3], n	6	0	0	4	0	0
Seizures, n	5	1	0	10	4	0
Cutaneous adverse events (dermatitis, allergic rash, alopecia), n	3	0	0	1	0	0

*1 patient in Arm B did not receive any study drug.

[1] The criteria were solely based on hematological toxicities that were observed while the patients were on treatment. To continue TMZ after a drug-free week, the following conditions had to be met: Absolute neutrophil count [counts/µl] (ANC) ≥ 1.5 x 10^3, Lymphocytes [counts/µl] (L) ≥ 500, Platelets [counts/µl] (P) ≥ 1 x 10^5, Hb [g/dl] ≥ 9. Dose adjustments were performed in dose levels. A dose level was defined as a step of 30 mg/m^2 in Arm A and as a step of 20 mg/m^2 in Arm B. Patients in Arm A started with a initial dose of 120 mg/m^2. Patients in Arm B started with an initial dose of 80 mg/m^2. If the following conditions were met throughout the first cycle, the dose was escalated in each arm as follows: ANC ≥ 2 x 10^3, L ≥ 800, P ≥ 1 x 10^5, Hb ≥ 11 [g/dl]; Dosage for second cycle Arm A: 150 mg/m^2, Arm B: 100 mg/m^2. Further dose escalations beyond 150 mg/m^2 in Arm A and beyond 100 mg/m^2 in Arm B were not allowed. TMZ administration should be interrupted if any of the following toxicities occured during a treatment week: ANC < 1.0 x 10^3, L ≥ 250, P < 5 x 10^4. TMZ administration should also be interrupted if non-haematological toxicities of CTC grade IV occur or if non-haematological toxicities of CTC grade III persist longer than 14 days. The occurrence of two of the following toxicities in line I or one the following toxicities in line II either in a treatment week or in a drug-free week necessitated a dose reduction in the following cycle: Line I (Reduction of dose levels -1) 1.0 x 10^3 < ANC < 1.5 x 10^3, 400 < L < 500, P 4 x 10^4 < P < 8 x 10^4; Line II (Reduction of dose levels -2) 0.5 x 10^3 < ANC < 1.0 x 10^3, 250< L < 400, 1x10^4 <

P $<4x10^4$. Treatment could be withheld up to 8 weeks after the last intake of TMZ. Interruption for more than 8 weeks due to hematologic toxicity was defined as treatment failure because of hematological toxicity, leading to withdrawal of the patient from study treatment.

[2] If toxicities occurred (as outlined above), prophylactic treatment with aciclovir as well as trimethoprim and sulfamethoxazol should be started depending on the lymphocyte counts and concomitant steroid medication: Lymphocytes [counts/µl] (L) $\leq$ 500 / µl, concomitant steroid medication: Aciclovir 5mg/kg t.i.d., Sulfamethoxazol 800 mg & Trimethoprim 160 mg b.i.d. once a week; L $\leq$ 500 / µl, no concomitant steroid medication: Sulfamethoxazol 800 mg & Trimethoprim 160 mg b.i.d. once a week; L > 500/µl, concomitant steroid medication: Sulfamethoxazol 800 mg & Trimethoprim 160 mg: b.i.d. once a week; L > 500/µl, no concomitant steroid medication: Optional.

[3] Antiemetics were applied to prevent nausea and vomiting during study treatment. The following drugs were recommended: ondansetron 4 mg, tropisetron 5 mg, 20 drops metoclopramide, domperidon 10 mg.

Supplementary Table 2. Outcome by *MGMT* promoter methylation status and treatment arm.

	MGMT-unmethylated glioblastoma			MGMT-methylated glioblastoma			
Arm A	Patients	Events	Time in months (95% CI)	Patients	Events	Time in months (95% CI)	p
Median TTF	28	27	1.8 [1.6; 2.9]	24	22	1.9 [1.7; 5.7]	0.164
Median survival from first study drug administration	28	25	6.6 [5.1; 11.5]	24	17	13.0 [9.3-17.9]	0.040
			Rate in % (95% CI)			Rate in % (95% CI)	
PFS-6	28	26	7 [1; 20]	22	16	29 [13; 49]	0.041
Survival rate at 12 months from first study drug administration	26	19	27 [12; 45]	19	8	59 [34; 77]	0.064
Arm B	Patients	Events	Time in months (95% CI)	Patients	Events	Time in months (95% CI)	p
Median TTF	31	29	1.9 [1.8; 1.9]	22	20	6.3 [2.0; 9.3]	0.0015
Median survival from first study drug administration	31	26	8.1 [6.1; 10.9]	22	19	12.1 [9.4; 21.5]	0.0065
			Rate in % (95% CI)			Rate in % (95% CI)	
PFS-6	30	28	7 [1; 19]	22	11	50 [28; 68]	0.0003
Survival rate at	27	22	19 [7; 35]	22	11	50 [28; 68]	0.016

12 months from first study drug administration							

Abbreviations: CI, confidence interval; PFS, progression-free survival; TTF, time to treatment failure.

Supplementary Table 3. Outcome by interval from last TMZ administration and *MGMT* promoter methylation status.

	Last TMZ < 2 months			Last TMZ ≥ 2 months			
All patients	Patients	Events	Time in months (95% CI)	Patients	Events	Time in months (95% CI)	p
Median TTF	40	39	1.8 [1.7; 2.0]	65	59	2.0 [1.8; 3.8]	0.0013
Median survival from first study drug administration	40	34	9.3 [7.3; 11.6]	65	53	10.7 [7.9; 12.5]	0.032
			Rate in % (95% CI)			Rate in % (95% CI)	
PFS-6	39	34	13 [5; 25]	63	47	26 [16; 37]	0.079
Survival rate at 12 months from first study drug administration	34	25	27 [14; 43]	60	35	42 [29; 54]	0.137
Arm A	Patients	Events	Median time, months (95% CI)	Patients	Events	Median time, months (95% CI)	p
Median TTF	20	20	1.8 [1.5; 2.3]	32	29	2.0 [1.8; 4.2]	0.024
Median survival from first study drug administration	20	17	8.5 [3.2; 12.0]	32	25	12.1 [6.4; 16.1]	0.074
			Rate in % (95% CI)			Rate in % (95% CI)	
PFS-6	20	18	10 [2; 27]	30	24	22 [9; 38]	0.253

Survival rate at 12 months from first study drug administration	17	13	25 [8; 46]	28	14	50 [31; 67]	0.069
Arm B	Patients	Events	Median time, months (95% CI)	Patients	Events	Median time, months (95% CI)	p
Median TTF	20	19	1.9 [1.8; 2.6]	33	30	2.0 [1.8; 5.5]	0.04
Median survival from first study drug administration	20	17	11.5 [5.7; 13.7]	33	28	10.6 [6.9; 12.6]	0.25
			Rate in % (95% CI)			Rate in % (95% CI)	
PFS-6	19	16	16 [4; 35]	33	23	30 [16; 46]	0.21
Survival rate at 12 months from first study drug administration	17	12	30 [11; 51]	32	21	34 [19; 51]	0.72
	Last TMZ < 2 months			Last TMZ ≥ 2 months			
***MGMT* promoter unmethylated**	Patients	Events	Time in months (95% CI)	Patients	Events	Time in months (95% CI)	p
Median TTF	29	28	1.9 (1.8; 2.0)	30	28	1.8 (1.8; 3.7)	0.30
Median survival from first study	29	25	8.5 [5.7; 12.0]	30	26	6.9 [5.6; 10.3]	0.76

drug administration							
			Rate in % (95% CI)			Rate in % (95% CI)	
PFS-6	28	26	7 [1; 20]	30	28	7 [1; 19]	0.47
Survival rate at 12 months from first study drug administration	25	18	28 [13; 46]	28	23	18 [7; 34]	0.19
Arm A	Patients	Events	Median time, months (95% CI)	Patients	Events	Median time, months (95% CI)	p
Median TTF	13	13	1.8 [1.5; 3.3]	15	14	1.8 [1.0; 3.7]	0.55
Median survival from first study drug administration	13	12	8.5 [2.9; 12.6]	15	13	6.4 [5.1; 12.1]	0.92
			Rate in % (95% CI)			Rate in % (95% CI)	
PFS-6	13	12	8 [0.5; 29]	15	14	7 [0.4; 26]	0.46
Survival rate at 12 months from first study drug administration	12	9	25 [6; 51]	14	10	29 [9; 53]	0.42
Arm B	Patients	Events	Median time, months (95% CI)	Patients	Events	Median time, months (95% CI)	p
Median TTF	16	15	1.9 [1.6; 1.9]	15	14	1.8 [1.7; 3.7]	0.53
Median	16	13	9.1 [4.1; 13.7]	15	13	8.0 [3.8; 10.6]	0.42

survival from first study drug administration							
			Rate in % (95% CI)			Rate in % (95% CI)	
PFS-6	15	14	7 [0.4; 26]	15	14	6.7 [0.4; 26]	1.00
Survival rate at 12 months from first study drug administration	13	9	31 [10; 56]	14	13	7 [0.5; 28]	0.05
	Last TMZ < 2 months			Last TMZ ≥ 2 months			
***MGMT* promoter methylated**	Patients	Events	Time in months (95% CI)	Patients	Events	Time in months (95% CI)	p
Median TTF	11	11	1.8 (1.4; 7.3)	35	31	5.5 (2.0; 9.3)	0.01
Median survival from first study drug administration	11	9	11.3 (3.1; 13.0)	35	27	16.1 (9.8; 21.5)	0.02
			Rate in % (95% CI)			Rate in % (95% CI)	
PFS-6	11	8	27 [7; 54]	33	19	44 [27; 59]	0.15
Survival rate at 12 months from first study drug administration	9	7	23 [3; 52]	32	12	63 [44; 77]	0.01

Arm A	Patients	Events	Median time, months (95% CI)	Patients	Events	Median time, months (95% CI)	p
Median TTF	7	7	1.7 [1.2; 1.8]	17	15	5.5 [1.8; 9.7]	0.01
Median survival from first study drug administration	7	5	9.3 [3.1; 13.0]	17	12	16.1 [6.8; 19.0]	0.03
			Rate in % (95% CI)			Rate in % (95% CI)	
PFS-6	7	6	14 [0.6; 46]	15	10	36 [14; 59]	0.12
Survival rate at 12 months from first study drug administration	5	4	21 [1; 60]	14	4	72 [42; 89]	0.01
Arm B	Patients	Events	Median time, months (95% CI)	Patients	Events	Median time, months (95% CI)	p
Median TTF	4	4	5.5 [1.8; 9.2]	18	16	6.3 [2.0; 9.9]	0.37
Median survival from first study drug administration	4	4	11.5 [9.4; 17.4]	18	15	14.5 [9.0; 21.8]	0.26
			Rate in % (95% CI)			Rate in % (95% CI)	
PFS-6	4	2	50 [6; 84]	18	9	50 [26; 70]	1.00
Survival rate at 12 months from first	4	3	25 [0.8; 67]	18	8	56 [31; 75]	0.11

study drug administration							

Abbreviations: CI, confidence interval; PFS, progression-free survival; TMZ, temozolomide; TTF, time to treatment failure.

Supplementary Table 4. Adherence to Mini Mental Status data collection over the course of the study.[1]

			Visit done	Patients with test: n / %					
				DONE		NOT DONE		MISSING	
Test	**Visit**	**Visit Cycle**							
Mini Mental Status	**Baseline visit**	**1**	105	100	95.24	5	4.76	.	.
	Treatment phase - Every 4 weeks	**1**	94	85	90.43	9	9.57	.	.
		2	73	60	82.19	12	16.44	1	1.37
		3	33	29	87.88	4	12.12	.	.
		4	31	30	96.77	1	3.23	.	.
		5	21	21	100.00	.	.	.	.
		6	20	18	90.00	2	10.00	.	.
		7	16	16	100.00	.	.	.	.
		8	14	13	92.86	1	7.14	.	.
		9	11	11	100.00	.	.	.	.
		10	9	9	100.00	.	.	.	.
		11	7	6	85.71	1	14.29	.	.
		12	6	6	100.00	.	.	.	.
		13	2	2	100.00	.	.	.	.
		14	1	1	100.00	.	.	.	.
		15	1	1	100.00	.	.	.	.
		16	1	1	100.00	.	.	.	.
		17	1	1	100.00	.	.	.	.
		18	1	1	100.00	.	.	.	.
		19	1	1	100.00	.	.	.	.
		20	1	1	100.00	.	.	.	.
	End of study medication visit	**1**	102	67	65.69	28	27.45	7	6.86
	Follow-up visit (after treatment failure),	**1**	55	31	56.36	23	41.82	1	1.82
		2	41	27	65.85	13	31.71	1	2.44
		3	26	20	76.92	6	23.08	.	.

			Visit done	Patients with test: n / %					
				DONE		NOT DONE		MISSING	
	thereafter in 8-weekly intervals	4	20	13	65.00	7	35.00	.	.
		5	12	8	66.67	4	33.33	.	.
		6	7	5	71.43	2	28.57	.	.
		7	3	3	100.00	.	.	.	.
		8	3	2	66.67	1	33.33	.	.
		9	1	.	.	1	100.00	.	.

Supplementary Table 5. Treatment effect (Arm B versus Arm A) on EORTC-QLQ-C30 and –BN20 scales evaluated at timepoint 90 days[1].

Questionnaire	Scale	Effect	95% CI	p value
EORTC QLQ-C30	Appetite loss	-5.08	[-14.87; 4.71]	0.3082
	Cognitive functioning	-1.91	[-12.18; 8.36]	0.7142
	Constipation	-0.58	[-11.04; 9.89]	0.9135
	Dyspnoea	-1.46	[-10.48; 7.56]	0.7501
	Emotional functioning	-4.11	[-12.87; 4.65]	0.3568
	Fatigue	-4.55	[-14.21; 5.10]	0.3536
	Financial difficulties	-5.82	[-14.82; 3.18]	0.2038
	Global health status	0.02	[-8.38; 8.43]	0.9954
	Insomnia	-3.39	[-12.83; 6.06]	0.4811
	Nausea and vomiting	2.47	[-3.99; 8.94]	0.4522
	Pain	4.36	[-5.90; 14.62]	0.4036
	Physical functioning	-4.73	[-13.79; 4.32]	0.3044
	Role functioning	-1.26	[-13.58; 11.05]	0.8400
	Social functioning	-2.16	[-11.55; 7.23]	0.6504
EORTC QLQ-BN20	Bladder control	1.72	[-9.96; 13.40]	0.7717
	Communication deficit	1.42	[-9.70; 12.55]	0.8014
	Drowsiness	-6.79	[-16.17; 2.60]	0.1555
	Future uncertainty	-7.24	[-16.72; 2.23]	0.1335
	Hair loss	-0.58	[-9.06; 7.90]	0.8928
	Headaches	11.83	[1.48; 22.18]	0.0253
	Itchy skin	0.20	[-8.01; 8.40]	0.9626
	Motor dysfunction	-3.37	[-13.81; 7.07]	0.5255
	Seizures	6.04	[-3.15; 15.22]	0.1969
	Visual disorder	5.24	[-1.77; 12.24]	0.1423

[1]Estimates of expected values of treatment differences at timepoint 90 days, obtained from a generalized linear mixed model incorporating baseline score, treatment group, days after randomization with time/treatment and time/baseline interactions.

Supplementary Table 6. Absolute changes of (0-100) score points of quality of life (as assessed by the EORTC-QLQ-C30 and -BN20 scales) within one treatment cycle of 28 days in Arms A and B, along with local p-values for difference in slopes.[1]

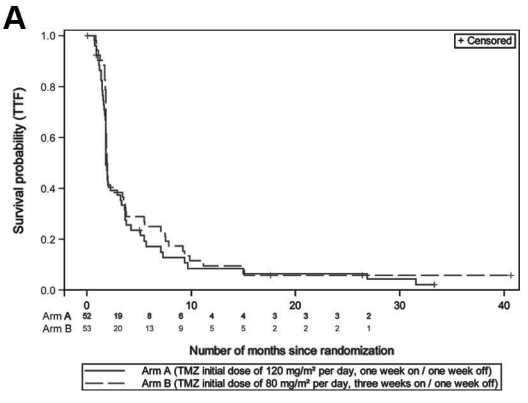
Questionnaire	Scale	Arm A	Arm B	p value
EORTC QLQ-C30	Appetite loss	0.130	0.821	0.054
	Cognitive functioning	0.270	0.984	0.055
	Constipation	-1.02	-.396	0.110
	Dyspnoea	0.093	0.422	0.316
	Emotional functioning	0.149	0.731	0.040
	Fatigue	0.124	1.118	0.005
	Financial difficulties	-.021	-.201	0.586
	Global health status	0.362	0.301	0.832
	Insomnia	0.156	0.362	0.647
	Nausea and vomiting	0.006	-.198	0.382
	Pain	-.364	0.795	0.001
	Physical functioning	0.310	1.142	0.007
	Role functioning	0.321	0.830	0.159
	Social functioning	0.660	1.167	0.119
EORTC QLQ-BN20	Bladder control	0.141	0.655	0.133
	Communication deficit	-.050	0.886	0.017
	Drowsiness	0.494	1.119	0.116
	Future uncertainty	0.300	1.199	0.003
	Hair loss	0.009	0.296	0.362
	Headaches	-.099	0.693	0.039
	Itchy skin	0.147	-.058	0.566
	Motor dysfunction	0.519	0.872	0.260
	Seizures	0.057	0.324	0.447
	Visual disorder	-.019	0.580	0.037

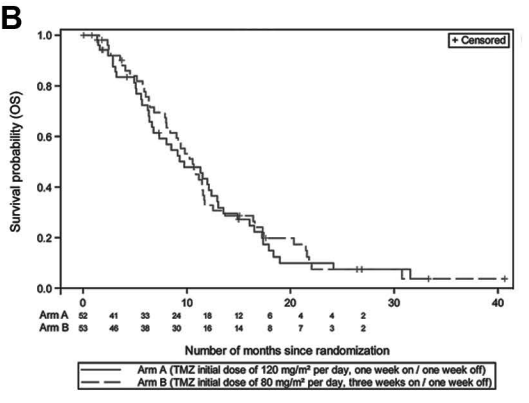
[1]Estimates of treatment by time interaction parameters in generalized linear mixed model incorporating baseline score, treatment group, days after randomization with time/treatment and time/baseline interactions.

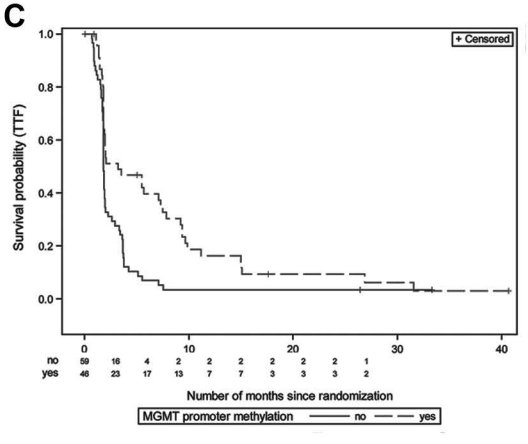
Supplementary Table 7. Adherence to EORTC-QLQ-BN20 and –C30 data collection over the course of the study.[1]

			Visit done	Patients with test: n / %					
				DONE		NOT DONE		MISSING	
Test	**Visit**	**Visit Cycle**	105	101	96.19	3	2.86	1	0.95
EORTC QLQ-C30/-BN20	**Baseline visit**	**1**							
	Treatment phase – every 8 weeks	**1**	86	75	87.21	7	8.14	4	4.65
		2	38	34	89.47	2	5.26	2	5.26
		3	23	21	91.30	1	4.35	1	4.35
		4	14	14	100.00	.	.	.	.
		5	11	11	100.00	.	.	.	.
		6	6	6	100.00	.	.	.	.
		7	1	1	100.00	.	.	.	.
		8	1	1	100.00	.	.	.	.
		9	1	1	100.00	.	.	.	.
		10	1	1	100.00	.	.	.	.
	End of study medication visit	**1**	102	66	64.71	26	25.49	10	9.80
	Follow-up visit (after treatment failure), thereafter in 8-weekly intervals	**1**	55	36	65.45	18	32.73	1	1.82
		2	41	30	73.17	11	26.83	.	.
		3	26	21	80.77	5	19.23	.	.
		4	20	17	85.00	3	15.00	.	.
		5	12	9	75.00	3	25.00	.	.
		6	7	5	71.43	2	28.57	.	.
		7	3	3	100.00	.	.	.	.
		8	3	3	100.00	.	.	.	.
		9	1	1	100.00	.	.	.	.

Figure 2


A
+ Censored
Survival probability (TTF)
1.0
0.8
0.6
0.4
0.2
0.0
0 10 20 30 40
Arm A 52 19 8 6 4 4 3 3 3 2
Arm B 53 20 13 9 5 5 2 2 2 1
Number of months since randomization
Arm A (TMZ initial dose of 120 mg/m² per day, one week on / one week off)
Arm B (TMZ initial dose of 80 mg/m² per day, three weeks on / one week off)


B
+ Censored
Survival probability (OS)
1.0
0.8
0.6
0.4
0.2
0.0
0 10 20 30 40
Arm A 52 41 33 24 18 12 6 4 4 2
Arm B 53 46 38 30 16 14 8 7 3 2
Number of months since randomization
Arm A (TMZ initial dose of 120 mg/m² per day, one week on / one week off)
Arm B (TMZ initial dose of 80 mg/m² per day, three weeks on / one week off)


C
+ Censored
Survival probability (TTF)
1.0
0.8
0.6
0.4
0.2
0.0
0 10 20 30 40
no 59 16 4 2 2 2 2 2 2 1
yes 46 23 17 13 7 7 3 3 3 2
Number of months since randomization
MGMT promoter methylation no yes

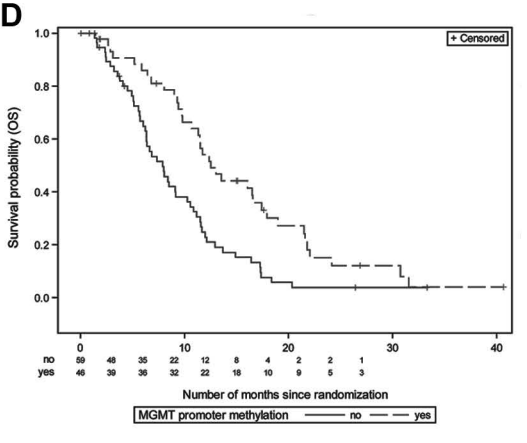

D
+ Censored
Survival probability (OS)
1.0
0.8
0.6
0.4
0.2
0.0
0 10 20 30 40
no 59 48 35 22 12 8 4 2 2 1
yes 46 39 36 32 22 18 10 9 5 3
Number of months since randomization
MGMT promoter methylation no yes

FIGURE 1 CONSORT CHART